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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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☐ *Additional inventors are being named on the ___ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

Terephthalamide derivatives as mimetics of the helical region of Bak peptide target Bcl-xL protein

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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☐ Drawing(s) *Number of Sheets*

☐ Application Data Sheet. See 37 CFR 1.76

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☐ Other (specify)

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Respectfully submitted,

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Date 02/19/04

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Terephthalamide derivatives as mimetics of the helical region of Bak peptide target Bcl-xL protein

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Abstract—A group of novel Bcl-xL/Bak antagonists, based on a terephthalamide scaffold, were designed to mimic the α-helical region of the Bak peptide. Good in vitro inhibition potencies in disrupting the Bak/Bcl-xL complex have been observed (terephthalamide **4**, $K_i = 0.78 \pm 0.07$ μM).
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Proteins in the Bcl-2 family play a critical role in determining the fate of a cell through the process of apoptosis.[1] Many oncogenic mutations, particularly those to p53, result in defects in DNA damage-induced apoptosis through a Bcl-2 dependent mechanism.[2] In addition, overexpression of Bcl-2 can inhibit the potency of many currently available anticancer drugs by blocking the apoptotic pathway.[3] Therefore, agents that directly mimic the death-promoting region BH3 domain of the pro-apoptotic subfamily of Bcl-2 proteins[4] are of potential therapeutic value.

The NMR-derived structure of the Bcl-xL/Bak BH3 domain complex indicates that the Bak peptide is an amphipathic α-helix that interacts with Bcl-xL by projecting hydrophobic side chains (Val^{74}, Leu^{78}, Ile^{81} and Ile^{85}) on one face of the α helix, into a hydrophobic cleft of Bcl-xL.[5] Several low molecular weight inhibitors of Bcl-2/Bcl-xL have been reported with the majority showing potency in the low μM range.[6] An alternative approach for identifying inhibitors is to design synthetic scaffolds that reproduce structural features of the BH3 helix region. We have previously reported functionalized terphenyls based on **1** as mimetics of the discontinuous binding epitopes of BH3.[7] However, the hydrophobicity of the terphenyls and their challenging syntheses prompted us to search for simpler scaffolds that could similarly mimic the side chain presentation on an α-helix. Herein, we report a group of novel Bcl-xL/Bak antagonists based on a terephthalamide scaffold, designed to mimic the α-helical region of the Bak peptide. Using a fluorescence polarization assay, we have observed high in vitro inhibition potencies in disrupting the Bcl-xL/Bak complex and a significant improvement of water solubility relative to the terphenyl derivatives.

The goal of our design was to maintain the similarity between the arrangement of the i, i+4, i+7 side chains of an α-helix and the substituents on 3, 2′, 2′- positions on terphenyl **1**,[7] while minimizing the structural complexity and increasing the solubility of the inhibitors. This strategy of simplifying a proven *proteomimetic* was accomplished by using terephthalamide **2** as the scaffold. The flanking phenyl rings in **1** were replaced by two functionalized carboxamide groups, which also retain a planar geometry due to the restricted rotation of the amide bonds. Figures 1A and B show, respectively, superimpositions of energy-minimized **2** on **1** (R1 = R2 = R3 = Me) with an RMS deviation value 0.34 Å and on the i, i+4, i+7 side chains of an α- helix with an RMS deviation value 1.03 Å, suggesting good stereochemical similarity between both pairs.[8]

A modular synthesis of terephthalamide derivative **4** is shown in Scheme 1. The 2-alkoxy group was introduced by *O*-alkylation and the 1-carboxylic acid was installed through Sandmeyer reaction and Stille coupling followed by Lemieux-Johnson and PDC oxidization. The amide bond formation steps were accomplished by using standard coupling conditions.

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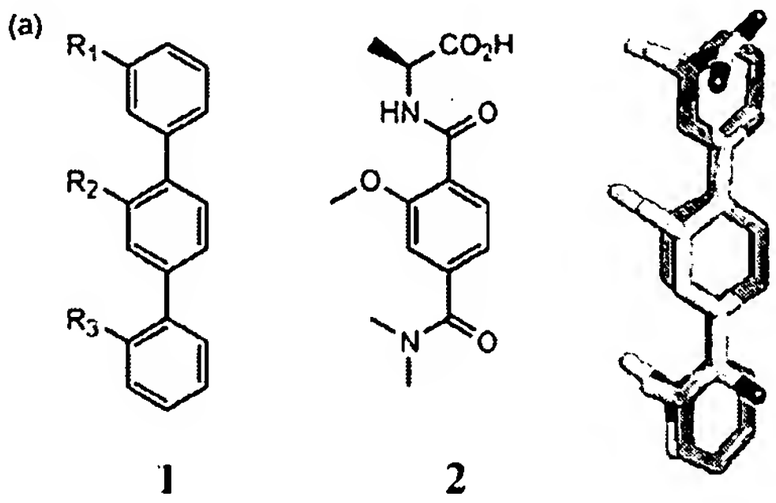
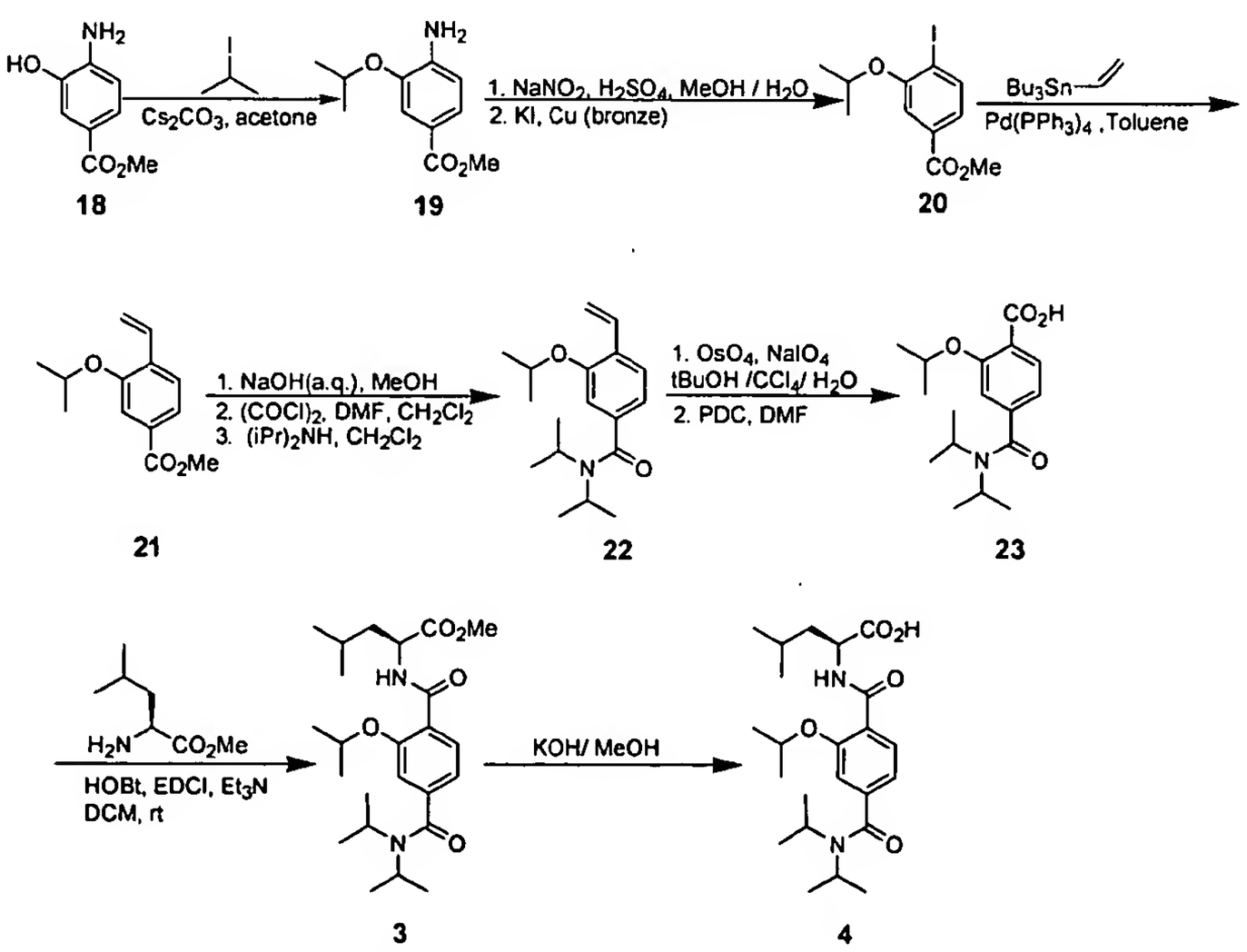


Figure 1. (A) The superimposition of terphenyl 1 on terephthalamide 2. (B) Stereo view of the superimposition of 2 on the i, i+4, i+7 positions of an α-helix.

Another conformational constraint in the molecule was imposed by an intramolecular hydrogen bond between the amide –NH and the alkoxy oxygen atom, to influence the position of the upper alkyl group (Fig. 2).[9] The intramolecular hydrogen bond was confirmed by variable temperature NMR, which showed very little change in the amide-NH resonance ($\delta = 0.54$ ppm) on heating ($\Delta\delta = 1.54$ ppm/K) or changing concentration.[10] As a comparison, 2-isopropylamino terephthalamide **17** showed both concentration (7.36 ppm, 0.5 M in $CDCl_3$; 6.58 ppm, 0.05 M in $CDCl_3$; 6.46 ppm, 0.005 M in $CDCl_3$, 298 K) and temperature ($\Delta\delta = 5.5$ ppb/K) dependence of the aniline proton, suggesting inter- rather than intramolecular hydrogen bonding.

Two strategies were used to orient the interacting *N*-alkyl group of the lower tertiary amide into the desired Z-conformation. In **3**, the problem was avoided by using identical substituents on the tertiary amide nitrogen. In a second series of derivatives (**5**, Scheme 2), steric differentiation of the substituents favored the placement of the isobutyl group in the Z-position (Fig. 3A). The conformation of **5** in solution was probed by ROESY and NOESY ^1H spectroscopy.[11] NOE cross peaks between H_b and the *ortho*-aryl protons were detected, while no significant NOE effect could be seen between H_a and the ortho- protons. However, correlations corresponding to the chemical exchange of H_a and H_b were observed in the ROESY experiment (Fig. 3B), which indicated there were two conformations of **5** existing in DMSO solution at 298 K. Furthermore, the signals of both H_a and H_b, which are split at room temperature, coalesced at 353 K. These combined experimental results suggest that both Z- and E-amide conformations are present with the Z-conformation being favored by 72% (from NMR integration), and by 8.01 kJ/mole in water solution (from MM2 energy minimization using Macromodel). The consequence of these constraints is that in low energy, accessible conformations of **5**, the three substituents project from one face of the terephthalamide scaffold in a manner analogous to the terphenyl helix mimetics (e.g., Fig. 1A).[7]

The binding affinity of the terephthalamide molecules for Bcl-xL was assessed by a fluorescence polarization assay using a fluorescently labeled 16-mer Bak-peptide (Fl-GQVGRQLAIIGDDINR-$CONH_2$).[5] Displacement of this probe through competitive binding of the



Scheme 1. Modular synthesis of terephthalamide derivatives **3** and **4**.

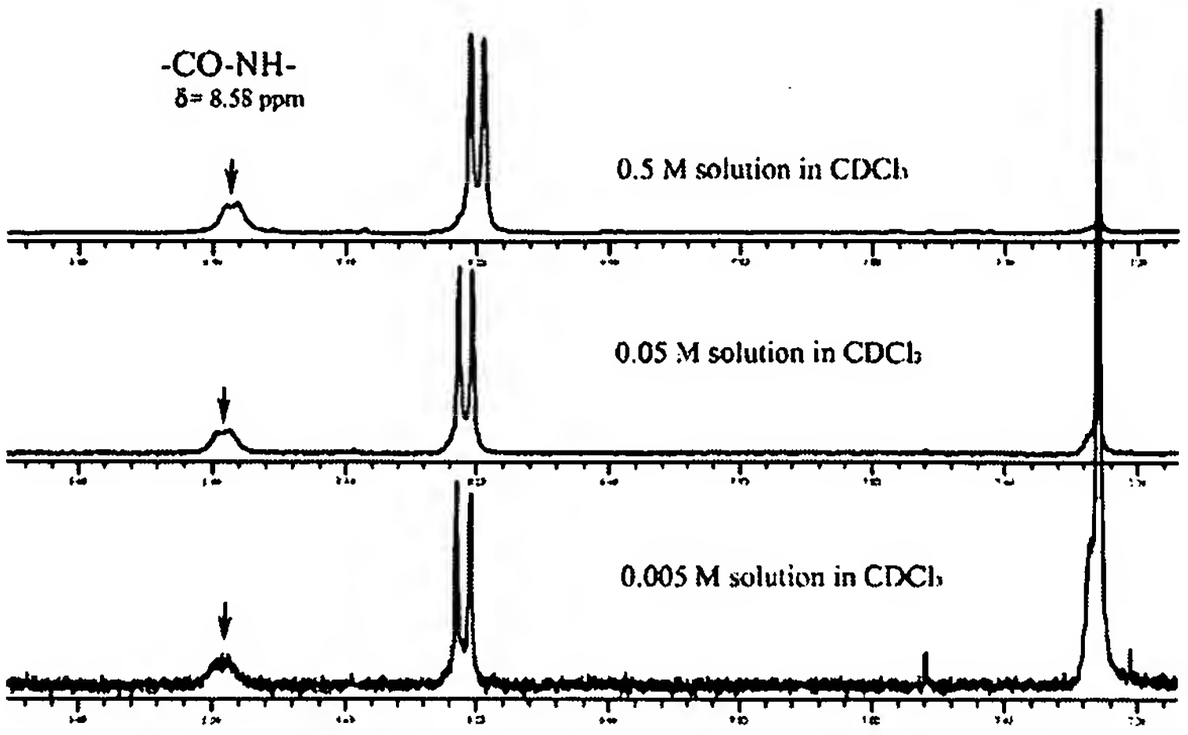
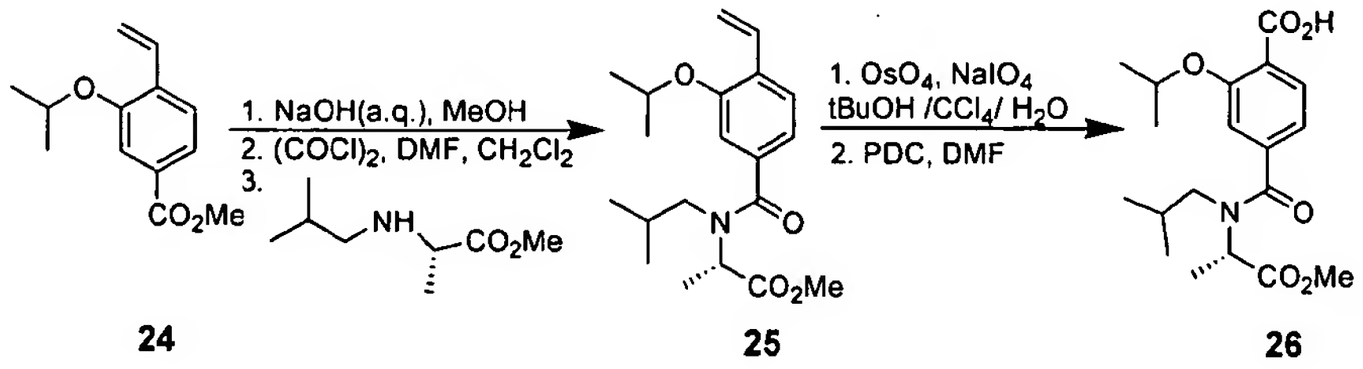
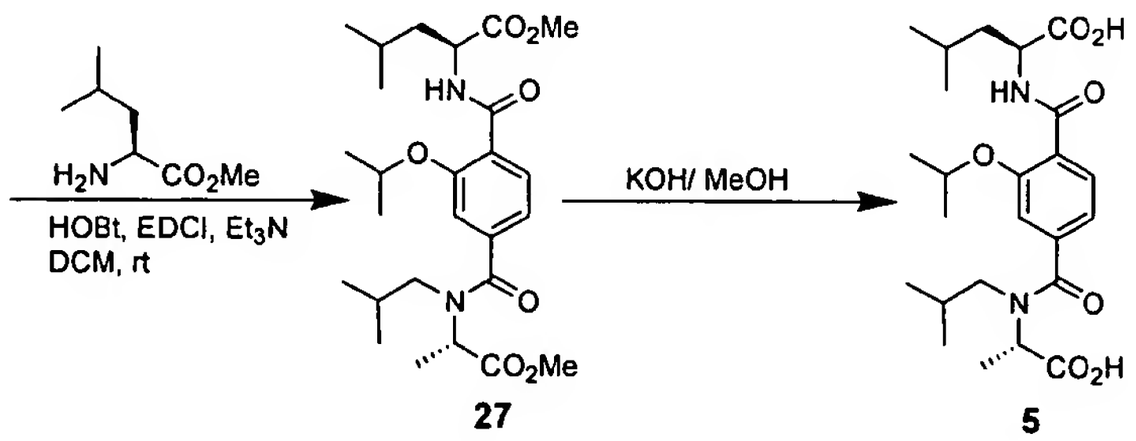


Figure 2. ^{1}H NMR spectra of low field region for **4** of different concentrations.





Scheme 2. Synthesis of terephthalamide derivative **5**.

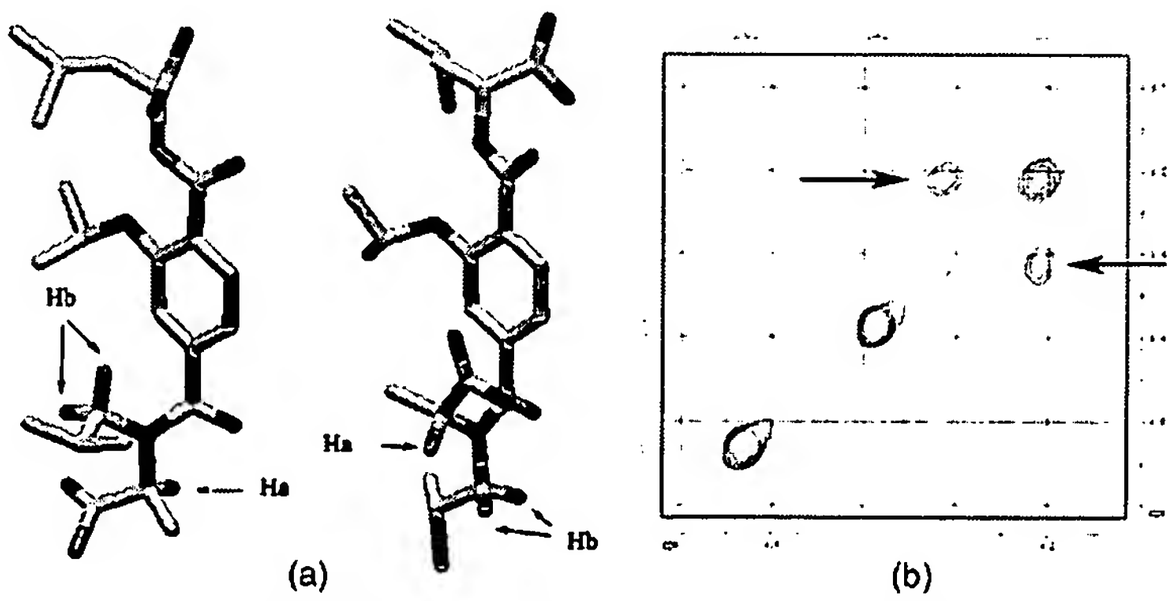


Figure 3. (A) Energy minimized Z- and E- isomers of **5**. (B) ROESY ^{1}H experiments showed cross peaks corresponding to chemical exchange of Ha.

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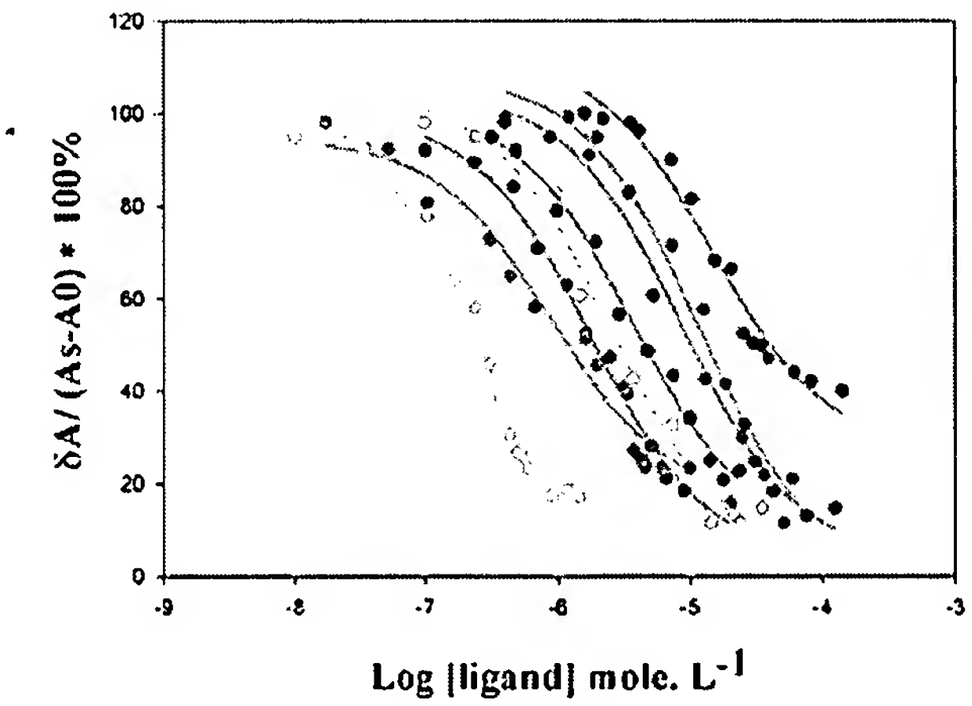
terephthalamide into the hydrophobic cleft of Bcl-xL would lead to a decrease in its fluorescence polarization, which in turn could be related to the known affinity of the 16-mer Bak peptide.[12] A series of terephthalamides with varied side chains was prepared. All the assays were carried out with 10^{-8} to 10^{-4} M terephthalamide solution in 10 mM PBS buffer (pH 7.4, 298 K) with less than 0.1% DMSO, indicating good solubility of terephthalamides in water. Figure 4 shows that terephthalamide **3** has good affinity for Bcl-xL with a K_i value of 0.78 ± 0.07 μM. By screening compounds with a range of side chains on the upper carboxamide, we found that the isobutyl group as the upper substituent provided the best inhibition results (**3**, **10**, **11**, **12**). The newly introduced stereogenic center in the terephthalamide did not affect the affinity, as seen by comparing **6** and **7**. The optimal alkoxy group in the 2-position of terephthalamide was found to be isopropoxy (**3**, **4**), which closely mimics the size of Leu^{78} of the Bak peptide; both larger (**8**, **9**) and smaller (**6**, **7**) substituents gave decreased affinities. The *N*,*N*-alkyl substituents on the lower carboxamide were shown to be crucial in the interaction since most of the affinity was lost when the symmetrical isopropyl groups on the amide nitrogen were replaced by other substituents (**5**, **13**, **14**, **15**). The importance of hydrophobic side chains was further confirmed by the weak binding of **16**, which lacks the key substituents. The 2-isopropylamino terephthalamide **17** showed affinity 4-fold less than its 2-isopropoxy analogue **4**, suggesting the intramolecular hydrogen bond in **4** helps to orient the side chain and in turn to enhance binding. These assay results confirmed that the terephthalamide derivatives retained the high in vitro affinity of the original terphenyl scaffold while reducing its complexity.

A computational docking study using Autodock™ lent support that the binding cleft for the BH3 domain of the Bak peptide on the surface of Bcl-xL is the target area for the synthetic inhibitors. Over 90% of the conformational search results showed the terephthalamide docked to this region. Figure 5 shows the overlay of the top-ranked docking result with the BH3 domain of the Bak peptide in the Bcl-xL/Bak complex, suggesting that the side chains of the terephthalamide scaffold have an analogous spatial arrangement to the three key alkyl side chains of the Bak peptide.

In conclusion, a novel family of Bcl-xL antagonists, based on a terephthalamide scaffold has been successfully



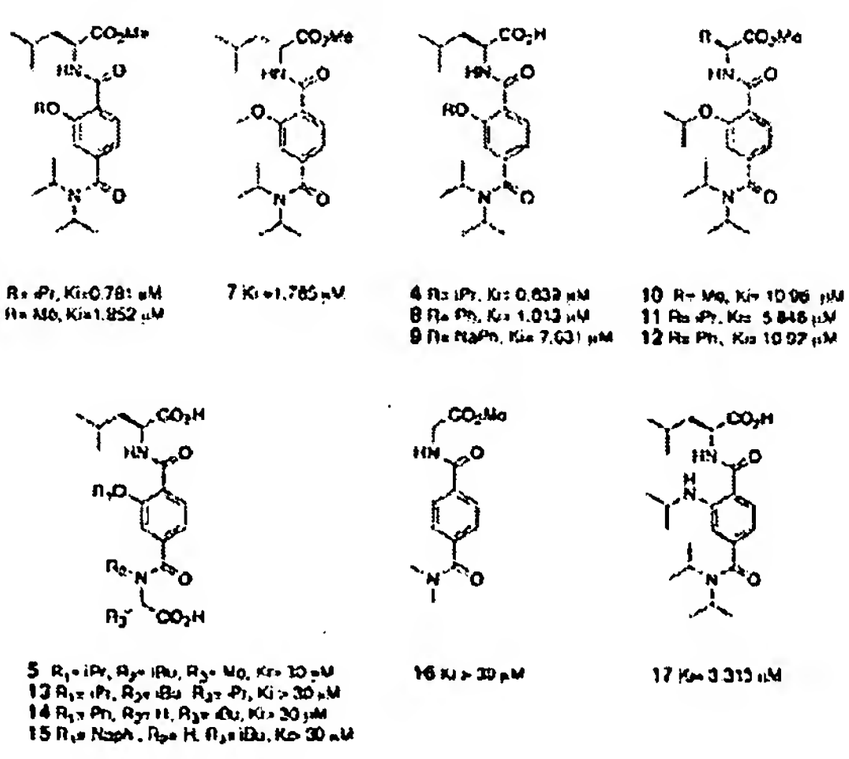


Figure 4. Results of the fluorescence polarization competition assay of terephthalamide derivatives as the antagonists of Bcl-xL/ Bak complex. ○ non-labeled Bak BH_3 peptide ● **3** ○ **6** ● **8** ● **9** ● **10** ● **16** ● **17**.

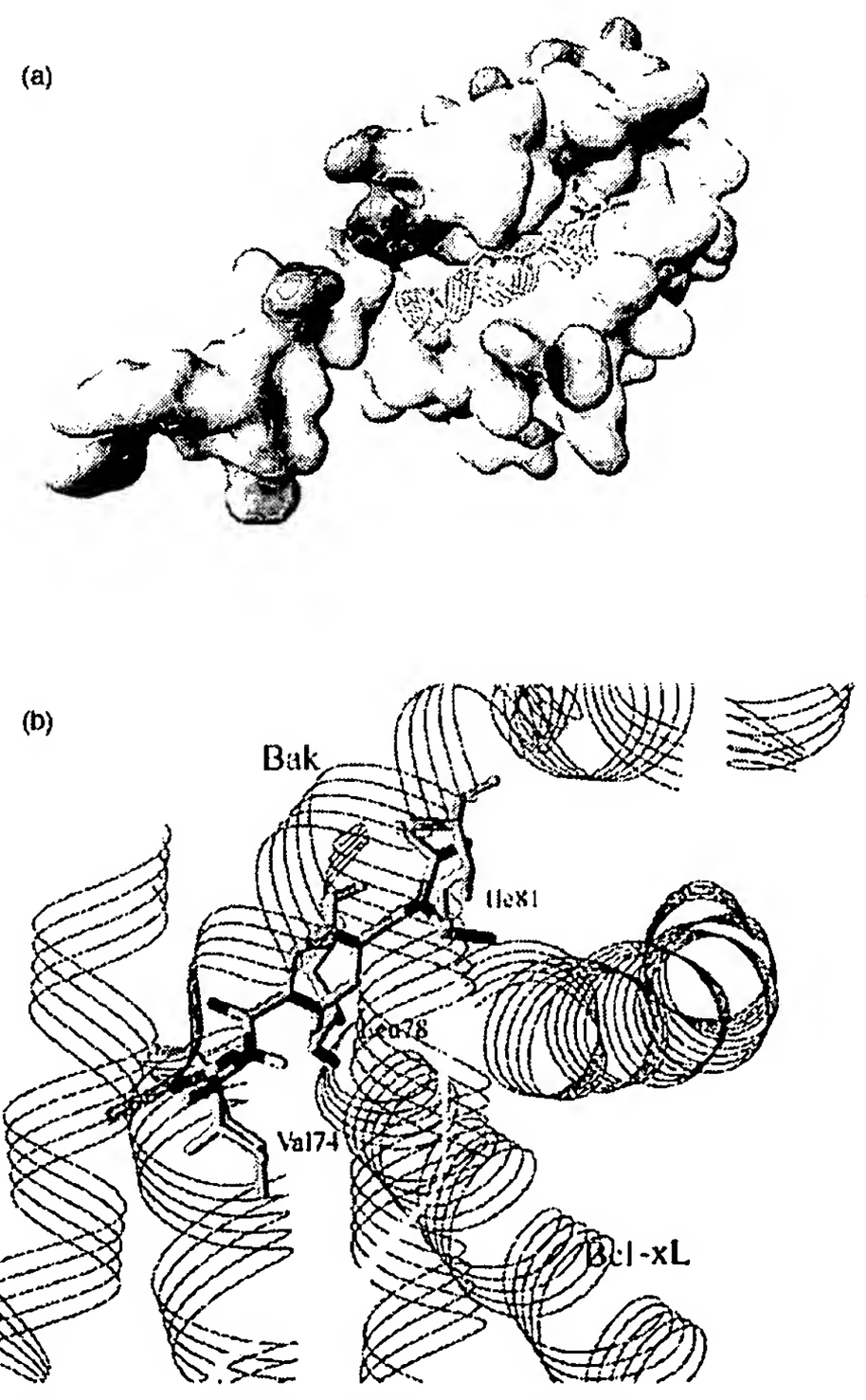


Figure 5. Result of the molecular-docking studies of **6** and Bcl-xL. (A) Full view. (B) Close up of an overlay of the highest-ranked binding mode and the Bak peptide. The key hydrophobic side chains of Bak are shown as stick representations.

developed. Systematic synthesis, conformational studies and fluorescence polarization binding assays were completed and sub- □M level in vitro affinities were observed.

Acknowledgements

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12. Under this assay condition, the unlabeled 16-mer Bak peptide gave a K_d of 120 nM (Kutzki, O. et al., *J. Am. Chem. Soc.* **2002**, 124, 11838, ref 10).